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TITLE: **Role of Sleep Deprivation in Fear Conditioning and Extinction: Implications for Treatment of PTSD**

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14. ABSTRACT PTSD is a growing concern for both active duty personnel and Veterans. Fear conditioning is implicated in the development of PTSD, while successful acquisition, consolidation, and recall of extinction memory are implicated in both the natural reduction of initial PTSD symptoms and as the mechanism underlying the most successful treatment for PTSD, Prolonged Exposure. This project is the first to examine the role of sleep and sleep loss in acquisition, consolidation, and generalization of extinction memory in humans. Our main finding is that sleep loss most strongly affects recall of extinction learning, and that the REM sleep stage is associated with ability to recall extinction as well as recall safety signal learning. These findings suggest that sleep plays a critical role in long term retention of fear inhibition processes, and efforts to support sleep and especially adequate REM during exposure therapy may enhance efficacy and reduce remission after treatment.				
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Introduction

PTSD is a growing concern for both active duty personnel and Veterans. Fear conditioning is implicated in the development of PTSD, while successful acquisition, consolidation, and recall of extinction memory are implicated in both the natural reduction of initial PTSD symptoms and as the mechanism underlying the most successful treatment for PTSD, Prolonged Exposure. In animal models, sleep deprivation has been shown to impair extinction memory. Indirect evidence in humans also supports that notion, but it has never been tested directly in humans. Some of the most ubiquitous and distressing symptoms of PTSD are insomnia and nightmares. The resultant sleep deprivation may actually serve to perpetuate the disorder by interfering with treatments designed to promote extinction memories. Before this hypothesis can be tested in clinical populations, however, well-controlled experimental studies need to establish the exact role of sleep deprivation in extinction acquisition, consolidation, and recall in humans. This study will do just that. This is a mixed-effects study designed to examine the impact of 36 hours TSD on fear conditioning and consolidation (Aim 1), as well as extinction memory acquisition, recall, and generalization (Aim 2). A total of 60 subjects will participate across 3 years. Following recruitment and screening, subjects will spend 4 nights and days in the laboratory: a) adaptation to the lab (Night/Day0); b) normal sleep followed by fear memory acquisition (Night/Day1); c) sleep or TSD followed by fear recall and extinction memory acquisition (Night/Day2); and d) sleep or TSD followed by a test of extinction recall and generalization (Night/Day3). Group1 will receive sleep prior to each testing day, Group2 will be sleep deprived prior to Day2, and Group3 will be sleep deprived prior to Day3.

Body

This report covers the entire project period. Specific activities include:

- a. Data Processing. As describe in a series of quarterly reports, we were finally able to continue spending on the project once the PI change was accepted, and hired data cleaners and a statistician to finalize all data cleaning and analysis (EMG and sleep variables).
- b. Data Analysis. We have completed our data analysis for our 3rd manuscript, which was submitted in November to Proceedings of the National Academy of Sciences.
- c. Dissemination. In addition to the manuscripts described below, Dr. Drummond has made several national and international presentations of our study findings. These include: a) an invited presentation at the Canadian Sleep Society meeting in Halifax, Nova Scotia in 2014; b) a departmental colloquium at Monash University in Melbourne, Australia in 2014; and c) an invited presentation at the Australian Centre for Posttraumatic Mental Health in 2014. Dr. Drummond also conducted a number of media interviews related to the Journal of Neuroscience paper described below. In 2015 Ms. Straus has made national presentations of our study findings at the Annual Meeting of the Associated Professional Sleep Studies in Seattle, Washington.
- d. Manuscripts. We have published two manuscripts, thus far from this study (reported last year), and have submitted a 3rd.
- e. In addition to the submission of the 3rd paper, we will also continue to work on a fourth paper to identify physiological sleep variables that impinge on extinction and safety recall (i.e. sympathetic responding via measures of heart rate variability during sleep).

Key Research Accomplishments

Data collection and analysis of all primary aims completed.

Manuscript 1:

Cognitive disruptions are commonly reported with PTSD and sleep deprivation, especially in tasks of attention. Within our sleep deprivation study we examined how sleep deprivation affects a novel measurement of attention in healthy humans, the 5-choice continuous performance task, and how analogous the effects of deprivation are to the homologues model in animals. These data helped validate the use of this task to assess sleep deprivation effects on cognitive performance using a cross-species task. These data suggest that this task may have translational utility in development of treatments for sleep-induced decrements in attention from animal models to proof-of-principle human trials.

van Enkhuizen, J., Acheson, D., Risbrough, V., Drummond, S., Geyer, M. A., & Young, J. W. (2014). Sleep deprivation impairs performance in the 5-choice continuous performance test: similarities between humans and mice. *Behavioural brain research*, 261, 40-48.

Several groups undergo extended periods without sleep due to working conditions or mental illness. Such sleep deprivation (SD) can deleteriously affect attentional processes and disrupt work and family functioning. Understanding the biological underpinnings of SD effects may assist in developing sleep therapies and [cognitive enhancers](#). Utilizing cross-species tests of attentional processing in humans and rodents would aid in mechanistic studies examining SD-induced inattention. We assessed the effects of 36 h of: (1) Total SD (TSD) in healthy male and female humans ($n = 50$); and (2) [REM](#) SD (RSD) in male C57BL/6 mice ($n = 26$) on performance in the cross-species 5-choice [continuous performance test](#) (5C-CPT). The 5C-CPT includes target trials on which subjects were required to respond and non-target trials on which subjects were required to inhibit from responding. TSD-induced effects on human psychomotor vigilance test (PVT) were also examined. Effects of SD were also examined on mice split into good and poor performance groups based on pre-deprivation scores. In the human 5C-CPT, TSD decreased hit rate and vigilance with trend-level effects on accuracy. In the PVT, TSD slowed response times and increased lapses. In the mouse 5C-CPT, RSD reduced accuracy and hit rate with trend-level effects on vigilance, primarily in good performers. In conclusion, SD induced impaired 5C-CPT performance in both humans and mice and validates the 5C-CPT as a cross-species translational task. The 5C-CPT can be used to examine mechanisms underlying SD-induced deficits in vigilance and assist in testing putative cognitive enhancers.

Manuscript 2:

This paper focuses on the main translational questions in the project. Specifically, this is the first paper to directly translate animal models of fear condition-sleep relationships into humans. Other human studies have examined extinction learning, but not the initial fear acquisition. This is critical to understanding PTSD, as without the initial fear acquisition, there would be no PTSD. We were also able to examine, for the first time in any species, the relationship between sleep and safety signals.

Marshall, A. J., Acheson, D. T., Risbrough, V. B., Straus, L. D., & Drummond, S. P. (2014). Fear conditioning, safety learning, and sleep in humans. *The Journal of Neuroscience*, 34(35), 11754-11760.

Fear conditioning is considered an animal model of Posttraumatic Stress Disorder (PTSD). Such models have shown fear conditioning disrupts subsequent Rapid Eye Movement Sleep (REM). Here, we provide a translation of these models into humans. Using the Fear Potentiated Startle (FPS) procedure, we examined the effects of fear conditioning and safety signal learning on subsequent REM sleep in healthy adults. We also examined the effects of changes in REM sleep on retention of fear and safety learning. Participants ($n=42$ normal controls) spent 3 consecutive nights in the lab. The first was an adaptation night. Following the second night, we administered a FPS procedure that included pairing a wrist shock with a threat signal and a safety signal never paired with a shock. The next day, we administered the FPS procedure again, with no wrist shocks to any stimulus, in order to measure retention of fear and safety. Canonical correlations assessed the relationship between FPS response and REM sleep. Results demonstrated increased safety signal learning during the initial acquisition phase was associated with increased REM sleep consolidation that night, with 28.4% of the variance in increased REM sleep consolidation from baseline accounted for by safety signal learning. Overnight REM sleep was, in turn, related to overnight retention of fear and safety learning, with

22.5% of the variance in startle retention accounted for by REM sleep. These data suggest sleep difficulties, specifically REM sleep fragmentation, may play a mechanistic role in PTSD via an influence on safety signal learning and/or threat-safety discrimination.

Note: This paper received considerable media attention as well as was the focus of a commentary in the Journal of Neuroscience.

Manuscript 3:

Laura D. Straus, Dean T. Acheson, Victoria B. Risbrough³, and Sean P.A. Drummond. Sleep Deprivation Disrupts Recall of Conditioned Fear Extinction in Healthy Humans.

Under Review at *Neuropsychopharmacology*

We have just submitted our 3rd manuscript to *Neuropsychopharmacology*, which we think will provide important new insights into how sleep affects fear extinction. In brief, we found that sleep deprivation before extinction training does not alter learning but does significantly reduce recall of extinction 24 hrs later. We did not find effects of sleep deprivation after extinction learning (before recall), suggesting that sleep before extinction training is the critical component to supporting long term fear inhibition (Figure 1). We also found that the amount of fear inhibition on day 3 (recall testing) was significantly associated with amount of REM consolidation, suggesting that REM sleep in particular may play an important role in retaining fear extinction memory (Figure 2).

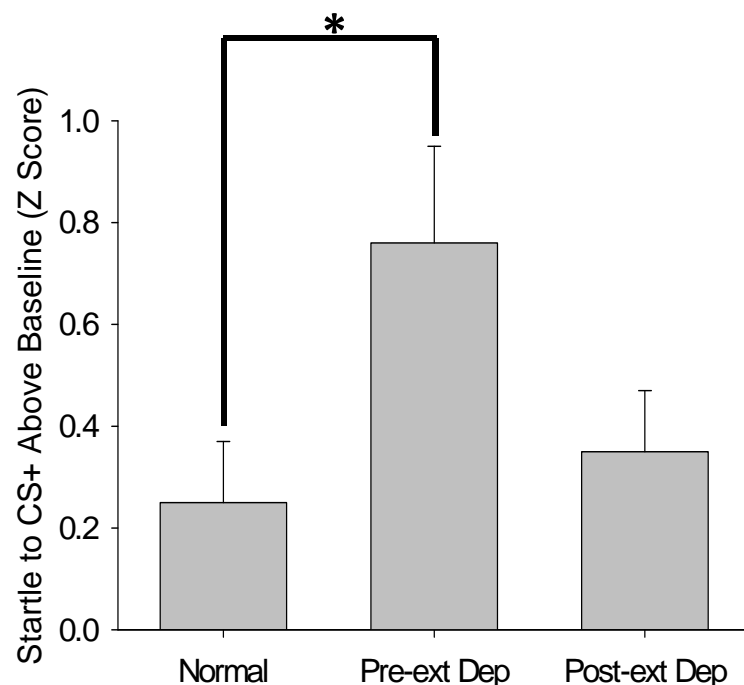


Figure 1: Pre-extinction training sleep deprivation reduces extinction recall. Standardized potentiated startle magnitudes by sleep group across the final recall session (day 3 of testing). $*=p<.05$ for a priori comparison with the normal sleep group. Normal = normal sleep group; Pre-ext Dep = pre-extinction sleep deprivation group, and Post-ext Dep = post-extinction sleep deprivation group. Note groups did not differ on level of fear conditioning, cued fear recall or extinction training sessions.

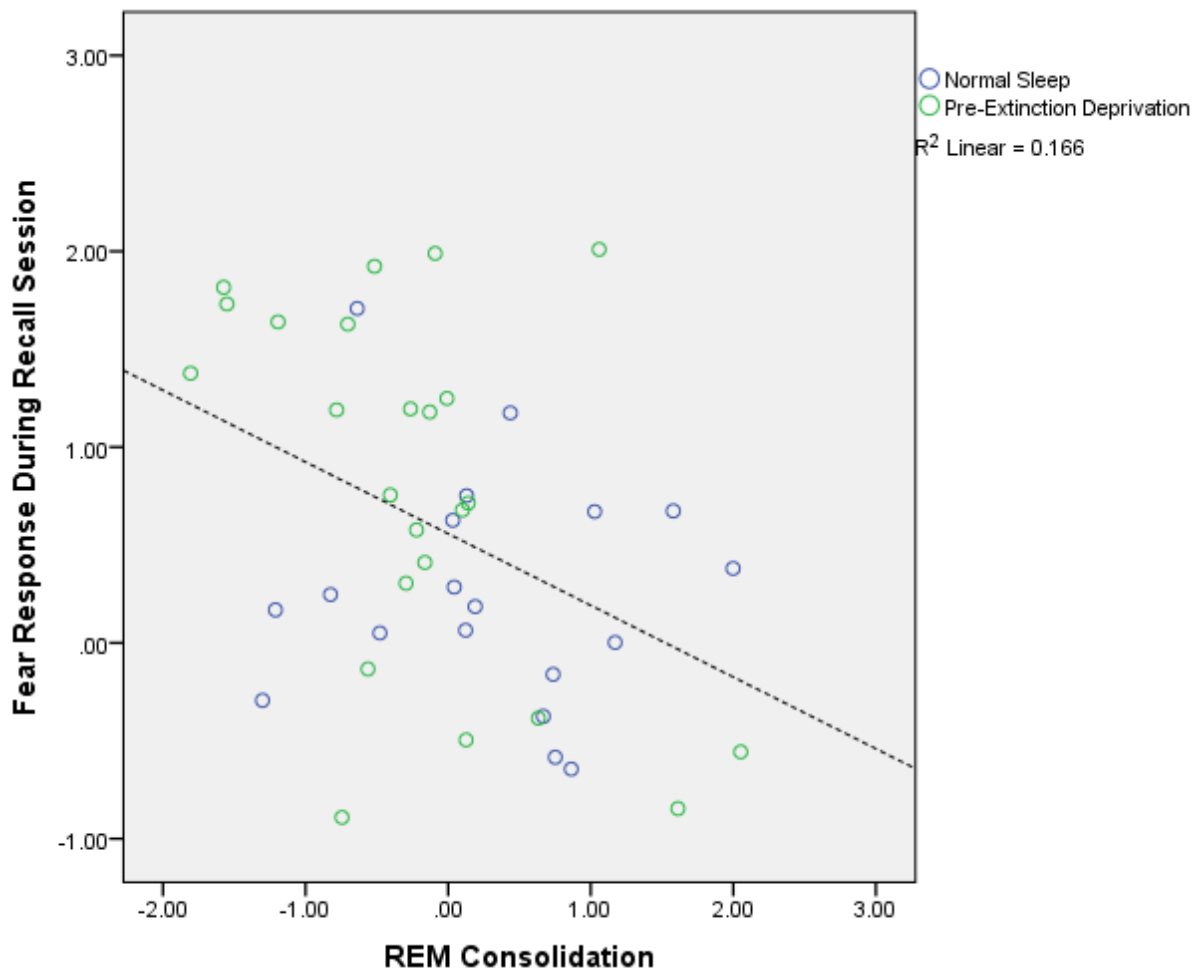


Figure 2: Relationship between REM sleep consolidation on Night 2 and extinction recall on Day 3, by group. Higher scores on REM consolidation on Night 2 corresponded to reduced fear responsiveness to the CS+ on Day 3, indicating enhanced extinction recall.

Conclusion/Reportable Outcomes

Over the project period, we were able to finalize data analysis for three manuscripts (2 now published and 1 under review) that complete the research aims of this project. We are also writing a fourth manuscript to understand how the sympathetic response, as measured by heart rate variability may also play a role in consolidation of fear learning and extinction processes during sleep.

There are two major implications of our findings. First, our finding that sleep deprivation before extinction learning suggests that adequate sleep is critical for retention of extinction recall. This finding has direct implications for exposure-based treatments for PTSD as well as preventing PTSD. In the treatment scenario, poor sleep, especially before exposure therapy sessions, may significantly impair the treatment efficacy of exposure therapy by inhibiting retention of an extinction acquired during the session. Second, it implies that poor sleep after trauma exposure may interfere with naturalist extinction learning, which could increase risk for development of PTSD. These data would suggest that prevention of PTSD may lie in part in supporting adequate sleep (and REM consolidation) after trauma exposure. Our data that REM consolidation may play a role in retention of extinction learning also supports the idea that sleep disturbances (e.g. circadian phase shifts) or drug treatments that suppress REM should be avoided after trauma exposure and certainly in subjects undergoing exposure therapy for PTSD. We are actively seeking funding to extend this research to understand how military operations that commonly effect sleep, e.g. circadian shifts or fragmented sleep with

loss of REM may affect extinction, as well as potential treatments (melatonin agonist treatments) to ameliorate operational effects on extinction.

References

See above

Appendices

Slides of presentations reported in section C.

Supporting Data

N/A